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INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE POLYMORPHISM AND OXIDATIVE STRESS IN CHRONIC HEPATITIS C VIRUS INFECTION.

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ABSTRACT

Cytokines like interleukin-1 receptor antagonist have an important role in the defense against viral infection. These cytokines are thought to play a central role in liver metabolism and in the immune response to viral agents. HCV infection is characterized by a systemic oxidative stress that is most likely caused by a combination of chronic inflammation, iron overload, liver damage, and proteins encoded by HCV. This work was planned in order to check the association of genetic polymorphism of interleukin-1 receptor antagonist (IL-1Ra) gene with chronic hepatitis C infection and its relation to oxidative stress markers.

The polymorphism analyzed, IL-1Ra genotype A1A2 showed lower significant frequencies in HCV patients when compared with controls (52.7% vs. 71.0%, p =0.006). This means that the genotype A1A2 might be considered a protective genotype against HCV infection. On the other hand, the frequency of A2A2 genotypes showed a higher significance in HCV patients than controls (15.5% vs. 8.1%, p= 0.02) and A1A1 genotype frequency was higher in patients when compared with controls (27.3% vs. 20.2%, p= 0.26),

Regarding to oxidative stress markers, in HCV patients showed significantly lower mean level of total antioxidant capacity (TAC) (p< 0.001), while these patients have significantly higher level of malonyldialdehyde (MDA) (p< 0.001). On the other hand, comparing cases-subgroups in terms of their assigned IL-1Ra genotypes, nitric oxide ($NO^{\cdot}$) mean level was significantly higher in patients having the A2A2 genotype than other patients.

Finally, the present study suggested that, the patients having the IL-1Ra A2A2 genotype were at higher risk for getting HCV infection and liver complications whereas those with A1A2 genotype seemed to be more protected against such infection. In addition, MDA, TAC and $NO^{\cdot}$ levels might be used as monitoring markers for oxidative stress in HCV cases with IL-1Ra A2A2 genotype.

Key words: Hepatitis C virus; chronic liver disease; IL-1 Receptor antagonist ; nitric oxide; total antioxidant capacity; malonyldialdehyde; Polymerase Chain Reaction.

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INTRODUCTION

According to World Health Organization (WHO) data, 3% of human population, i.e. approximately 170 million people, is infected with HCV. Chronically infected individuals serve as a source of transmission to others and are at risk for severe HCV related diseases, such as liver cirrhosis and hepatocellular carcinoma, during the decades after initial infection [**Brass *et al.*, (2006)**]. Therefore, it is important to estimate the demographic history of HCV infection, to predict the future burden of disease. Egypt is the highest prevalence of hepatitis C in the world and the national prevalence rate of HCV antibody positively has been estimated to be between 10-13% [**Mohamed (2004)**].

Cytokines are thought to have a central role in liver metabolism and in the immune response to viral agents [**Gramantieri *et al.*, (1999)**].These cytokines have an important role in defense against viral infection, indirectly through determination of the predominant pattern of the host response, and directly through inhibition of viral replication [**Koziel (1999)**]. In fact, serum levels of cytokines are elevated in patients with chronic hepatitis C, suggesting their role in inflammation of the liver [**Falasca *et al.*, (2006) and Gramenzi *et al.*, (2005)**].

The heritable differences in cytokine gene expression have been proposed as risk factors for the development of liver cirrhosis in chronic hepatitis C [**Poynard *et al.*, (2001)**]. Genes encoding interlukin-1 (IL-1) are located on the 430 kb region of chromosome 2q13-21, consisting of three homologous genes: IL-1A, IL-1β and interlukin-1 receptor antagonist (IL-1Ra) [**Nemetz *et al.*, (1999)**]. The IL-1 Receptor antagonist (IL-1Ra) is one member of the IL-1 family that represents an agent which binds to the same receptor on the cell surface as IL-1, and thus prevents IL-1 from sending a signal to that cell [**Bahr *et al*.,(2003)**].

In hepatitis C, more severe grades of inflammation and fibrosis are associated with a shift in the balance between the hepatic expression of interleukin IL-1β and interlukin

1-receptor agonist (IL-1Ra) towards more IL-1β activity [**Gramantieri *et al.*, (1999)**] and genetic polymorphism for both IL-1ß and IL-1Ra may increase the risk of HCV patients to develop cirrhosis [**Bahr *et al.*, (2003)**].

IL-1Ra contains an 86 bp variable number tandem repeat (VNTR) polymorphism in intron 2 [**Garcia-Gonzalez *et al.*, (2001)**]. These polymorphisms are located within the regulatory regions of the genes and have potential functional importance by modulating IL-1 protein production, and are related to the development of some diseases [**Moos *et al.*, (2001)**]. There were five alleles in humans, including Allele 1 (four repeats, 410 bp), allele 2 (two repeats, 240 bp), Allele 3 (five repeats, 500 bp), allele 4 (three repeats, 325 bp) and Allele 5 (six repeats, 595 bp) [**Zhang *et al.*, (2004)**].

HCV infection is characterized by a systemic oxidative stress that is most likely caused by a combination of chronic inflammation, iron overload, liver damage, and proteins encoded by HCV. The increased generation of reactive oxygen and nitrogen species, together with the decreased antioxidant defense, promotes the development and progression of hepatic and extra hepatic complications of HCV infection [**Choi *et al.*, (2004)**]. Progression of liver disease was found to be linked with factors including modification of the immune response [**Eggers *et al.*,(2006)**] and activation of proinflammatory cytokines both by virus C infection [**Castellano-Higuera *et al* , (2008)**].

Oxidative stress occurs due to discordance in balance between pro-oxidants and antioxidants. Normally, a rise in oxidative stress concomitantly enhances the anti - oxidative activity to protect the cell from damage. Chronic exposure to increased levels of oxidative stress may results in excess of reactive oxygen species within the hepatocytes [**Pessayre *et al*, (2001)**]. Oxidative stress can be described as a condition resulting from an uncontrolled increase in free oxygen radicals or an insufficiency in the antioxidant system under certain pathological states [**Sariçam *et al.*,(2005)**].

Hepatitis C leads to up-regulation of hepatic inducible nitric oxide synthase (iNOS) gene expression and given that liver inflammation with hepatocellular damage, and fibrosis, it is likely that NO could be an active mediator inducing liver injury and carcinogenesis [**Majano *et al.*, (2003)**].Liver tissue from HCV-infected patients was shown to express elevated levels of iNOS transcripts compared with non–HCV-infected patients [**Pârvu *et al.*, (2005)**].

SUBJECTS AND METHODS

This study was performed on 110 patients with hepatitis C virus (HCV). They were recruited from the Department of Tropical Medicine, Mansoura University Hospital, Egypt during the time of January 2009 to February 2010. These patients were 80 males and 30 females aged from 26.0 to 60.0 years. Their mean age were 43.3±6.4 years. They were all positive for HCV antibody and HCV RNA that was detected quantitively by real time PCR (RT- PCR). These cases include 43.0 (31.9%) of them gave a history of Bilharziasis while 26 (19.3%) were complicated with liver cirrhosis manifested with ascietis. The control subjects including 124 healthy blood donors were taken as controls. They did not suffer from any liver disorders or other infectious,

allergic or autoimmune diseases. They were in the form of 110 males and 14 females with age ranging between 17.0 - 42.0 years with a mean ±SD 26.0±5.7 years and a median age of 25.0 years (Table 1). An authorized approval was obtained from Mansoura University Scientific and Ethical Committees, in addition to an informed consent that was taken from all participants before the study.

Table (1): Demographic data of all studied HCV patients and controls.

Parameters	HCV patients (n=110)	Controls (n=124)
Age (years)	26.0 to 60.0	17.0 to 42.0
Mean ± SD	43.3 ± 6.4	26.0 ± 5.7
Median	44.0	25.0
Sex (M/ F)	80/30	110/14
Ascietis	26.0 (19.3%)	0.0 (0.0%)
Bilharziasis	43.0 (31.9%)	0.0 (0.0%)
Smoking	24.0 (17.8%)	0.0 (0.0%)

DNA extraction and amplification of IL-1Ra intron 2:

For all cases and controls, DNA was extracted and purified using the generation DNA purification capture column kits (Gentra system, USA). Amplification via PCR technique was carried out for IL-1Ra intron 2 contained the 86 bp VNTR. Each PCR was carried out in 25 µL reaction mixture containing 10 µl Master Mix (Fermentas, Germany), 8 µl PCR grade water, 2 µl IL-1fa primer, 2 µl IL-1ra primer (Bio Basic Inc., Canada) and 3 µl extracted DNA. Primer sequence was (F): 5'-TCCTGGTCTGCAGGTAA-3', (R): 5'-CTCAGCAACACTCCTAT-3' [**Kanemoto *et al.*,(2000)**].

PCR conditions were as follows: initial denaturation cycle of 95°C for 5 min followed by 35 cycles in the form of 94°C for 30 s (denaturation), 55°C for 30 s (annealing) and 72°C for 1 min (extension) with a final extension cycle of 5 min at 72°C. Amplified segment was analyzed using Agarose gel (3%) electrophoresis and photographed on ultraviolet transilluminator [**Zhang *et al.*, (2004)**].

Determination of biochemical markers related to Oxidative Stress:

Colorimetric determination of nitric oxide ($NO^{\cdot}$) was done using nitrite assay kits (Biodiagnostic, Cairo, Egypt) through the method described by [**Montgomery *et al.*, (1961)**] and determination of total antioxidant capacity (TAC) was done by using a commercially available kit (Biodiagnostic, Cairo, Egypt) through the method described by [**Koracevic *et al.*,(2001)**], while estimation of serum malonyldialdehyde (MDA) was carried out through thiobarbituric acid (TBA) test using the method described by [**Draper and Hadley, (1990)**].

STATISTICAL ANALYSIS

Statistical analysis was done using the statistical package of social science (SPSS) software version 13. Gene frequency was determined by the gene counting method. Comparison between groups was done using Chi square test ($\chi 2$) in addition to Odds ratio and 95% confidence intervals for the frequencies of studied alleles and genotypes; and student t-test for the numeric variables as oxidative stress markers. Probability (p) value was considered significant at a level ≤ 0.05 [**SPSS, 1999**].

RESULTS

In the present study, 4 alleles and 5 genotypes of IL-1Ra gene could be recognized among Egyptian cases with HCV infection and normal controls, including Allele 1 (four repeats, 410 bp), allele 2 (two repeats, 240 bp), Allele 3 (five repeats, 500 bp), allele 4 (three repeats, 325 bp) and Allele 5 (six repeats, 595 bp) (Figure 1).

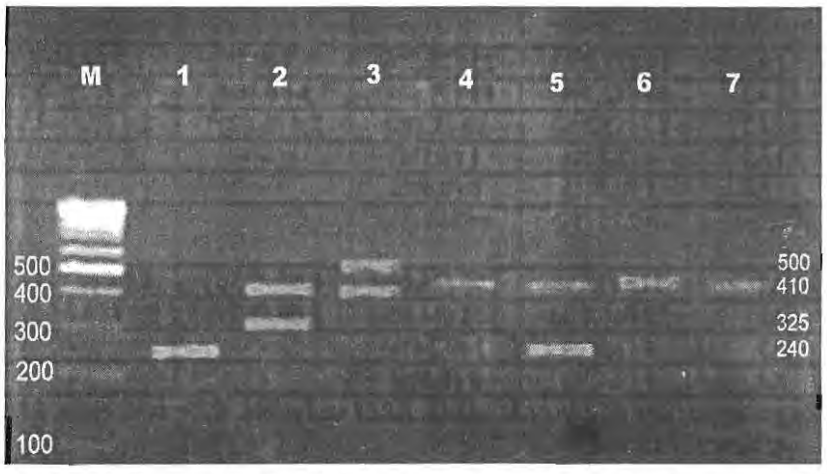

Fig (1): PCR product for IL-1Rª intron 2 gene polymorphism in HCV patients.

Lane (M): show DNA molecular mass marker 100 bp.

Lane (1): showed homozygote polymorphism of IL-1Ra A2/A2 genotype where A2 at 240 bp.

Lane (2): showed heterozygote polymorphism of IL-1Ra A1/A4 genotype where A1 at 410 bp and A4 at 325 bp.

Lane (3): showed heterozygote polymorphism of IL-1Ra A1/A3 genotype where A1 at 410 bp and A3 at 500 bp

Lane (4,6,7): showed homozygote polymorphism of IL-1Ra A1/A1 genotype where A1 at 410 bp .

Lane (5): showed heterozygote polymorphism of IL-1Ra A1/A2 genotype where A1 at 410 bp and A2 at 240 bp.

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that the genotype A1A2 might be considered a protective genotype against HCV infection.

On the other hand, the frequency of A1A1 and A2A2 homozygote genotype was higher in HCV patients than control group but is statistically insignificant (27.3% vs. 20.2% and 15.5% vs. 8.1% respectively, $p>0.02$) **Table (2).**

Table (2): Study of interleukin-1 receptor antagonist (IL-1Ra) in patients with HCV and controls.

IL-1Ra	Groups		$\chi 2$ (p)	OR (95% CI)
	HCV patients n=110 (%)	Control n=124 (%)		
Genotypes				
A1A1	30 (27.3%)	25 (20.2%)	1.3 (0.26)	1.5 (0.81-2.7)
A1A2	58 (52.7%)	88 (71.0%)	7.5 (0.006)*	0.4 (0.27-0.78)
A2A2	17 (15.5%)	10 (8.1%)	2.4 (0.02)*	2.1 (0.91-4.77)
A1A3	4 (3.6%)	1 (0.7%)	-	-
A1A4	1 (0.9%)	0 (0.0)%	-	-
Alleles	n=220 (%)	n=248 (%)		
A1	123 (55.9)	139 (56.0%)	0.01 (0.95)	0.99 (0.7-1.43)
A2	92 (41.8%)	108 (44.0%)	0.08 (0.78)	0.93 (0.65-1.35)
A3	4 (1.8%)	1 (0.34%)	-	-
A4	1 (0.4%)	0 (0.0%)	-	-

IL-1Ra genotypes are A1A1, A1A2, A2A2, A1A3 and A1A4. OR= odds ratio, 95% CI= 95% confidences interval,
$\chi 2$= Chi square test. p significant <0.05, (-) test is not applicable.*

Patients with HCV infection associated with ascietis and liver cirrhosis showed a higher frequency of A2A2 genotype with a high odds ratio of 3.4 (95% CI = 1.2-10.4) compared with the cases having HCV without cirrhosis or ascietis, while patients with a history of bilharziasis showed the same results of significantly lower frequency of A1A2 genotype and non-significantly higher frequency of the other genotypes A1A1 and A2A2 compared to controls **Table (3).**

Table (3): IL-1Ra genotypes in patients of HCV with Bilharziasis and Ascietis compared to controls.

	A1A1	A1A2	A2A2
Controls (n=124)	25 (20.2%)	88 (71.0%)	10 (8.1%)
Bilharziasis (n=43)	13 (37.1%)	13 (37.1%)	7 (20.0%)
$\chi 2$ (*p*)#	1.3 (0.3)	20.5 (<0.0001)**	1.5 (0.22)
OR (95% CI)	1.72 (0.78-3.8)	0.2 (0.1-0.4)	2.2 (0.8-6.3)
Ascitis (n=26)	5 (19.2%)	13 (50.0%)	6 (23.1%)
$\chi 2$ (*p*)	0.02 (0.87)	3.3 (0.06)	3.6 (0.05)*
OR (95% CI)	0.94 (0.32-2.7)	0.41 (0.2-0.98)	3.4 (1.2-10.4)*

*# Chi square test comparing cases subgroups with bilharziasis and ascities to controls, *p significant at 0.05 level, **p significant at <0.001 level.*

Table (4) showed a significant decrease of serum activities of total antioxidant capacity (TAC) of patients with HCV infections (1.10 ± 0.10 mM/L) compared to controls value (1.35 ± 0.46 mM/L) (*p*= <0.001).While these patients have highly significant value with malonyldialdehyde (15.23 ± 3.28 nmol/ml) compared to control group (7.92 ± 2.74 nmol/ml) (p= <0.001).

Table (4): Total antioxidant capacity, nitric oxide and malonyldialdehyde of all studied HCV cases and controls.

Parameters	HCV patients M ± SD	Controls M ± SD	*P* value <0.05
TAC (mM/L)	1.10 ± 0.10	1.35 ± 0.46	0.001**
MDA (nmol/ml)	15.23 ± 3.28	7.92 ± 2.74	0.001**
NO (µmol)	22.82 ± 9.26	20.56 ± 6.66	0.17

*TAC=Total Antioxidant Capacity, NO=Nitric Oxide, MDA=Malonyldialdehyde . Significant *p<0.05, highly significant **p<0.001.*

The mean level values of TAC was shown to affect cases carrying the IL-1Ra A1A2 genotype (1.13 ±0.09 vs. 1.63 ± 0.59, p= 0.01) while cases carrying the A1A1 and A1A2 genotypes showed significantly higher levels of MDA than controls (15.22 ± 2 13 vs. 8.46± 2.49, p=0.001) and (14.76 ± 3.58 vs. 8.72 ± 2.31, p=0.001) respectively. The mean level of NO˙ among HCV patients assigned IL-1Ra genotypes were significally elevated in patients having the A2A2 genotype followed by those having A1A2 and A1A1 genotypes (p=0.036) **Table (5).**

Table (5): Serum activities of TAC (mM/L), NO (µmol), and MDA (nmol/ml) of HCV infected patients and control groups regarding IL-1Ra genotypes.

Parameters and IL-1Ra genotypes	HCV patients M ± SD	Control M ± SD
TAC (mM/L)		
A1A1	1.13 ±.08	1.24 ± 0.56
A1A2*	1.13±.0.09	1.63 ± 0.59
A2A2	1.18 ± 0.01	1.32 ± 0.11
MDA (nmol/ml)		
A1A1**	15.22 ± 2.13	8.46 ± 2.49
A1A2**	14.76 ± 3.58	8.72 ± 2.31
A2A2	17.09 ± 2.33	7.83 ± 5.38
NO (µmol)		
A1A1	15.48 ± 3.44	22.63 ± 10.56
A1A2	22.23 ± 10.85	21.37 ± 5.85
A2A2#	29.64 ± 8.15	28.07 ± 7.55

*IL-1Ra genotypes are A1A1, A1A2 and A2A2. TAC=total antioxidant capacity, NO=nitric oxide, MDA=malonyldialdehyde. # NO concentration was significantly higher among cases with IL-1Ra A2A2 than A1A1 type, *p significant <0.05, **p significant <0.001.*

DISCUSSION

Cytokines play an important role in liver metabolism and in the immune response to viral agents [**Akpolat *et al.*, (2005)**]. In this study, 4 alleles and 5 genotypes of IL-1Ra gene could be recognized among Egyptian cases with HCV infection and normal controls. This genotype, A2A2 was the least frequent among Egyptian control subjects while the most frequent genotype was the heterozygosity genotype A1A2 that was significantly lower in patients with HCV compared to controls. On the other hand, homozygosity genotypes particularly of A2A2 was found higher among cases of HCV than controls mounting to a significant level among cases with ascietis due to liver cirrhosis. The same distribution was also found among cases of HCV associated with

bilharziasis. This may lead to speculate that homozygosity for the rare allele A2 is probably a risk genotype not only for liver affection with HCV and bilharziasis but also for the progression towards cirrhosis and ascietis. In contrast, heterozygosity genotype A1A2 seems to be probably protective against such affection.

Similarly, A2 allele was observed in about 10 to 30% of the Caucasian control population [**Bahr *et al.*, (2003)**]. Also, [**Wang *et al.*, (2003)**] found that the IL-1Ra A2 allele was less common in Japanese population (heterozygotes 5% to 9%; homozygotes, 0% to 2%). Regarding the homozygous genotype A1A1, it was found to be higher among Egyptian HCV cases (27.3%) compared to controls (20.2%), while [**Constantini *et al.*, (2002)**] found that IL-1RN A1/A1 genotype was found in 66% of Northern European Caucasoid adult HCV patients but only 45% in HCV patient from Poland. Also, [**Gramantieri *et al.*, (1999)**] found that among Italian population the IL-1Ra A2 allele was significantly more prevalent in patients with HCV-induced cirrhosis as compared to patients with chronic hepatitis C virus and controls. These points indicted that the role of IL-1Ra in the progression from chronic hepatitis to cirrhosis. In contrast, [**Wang *et al.*, (2003) and Tanaka *et al.*, (2003)**] stated that the IL-1RN A2A2 genotype had no association with liver disease progression, perhaps because it was less common in Japanese individuals (heterozygote 5% to 9%; homozygote, 0% to 2%).

In the present study we found that, no significant differences observed with respect to the rare IL-1Ra allele A3 and IL-1Ra allele A4 in Egyptian cases and controls. This observation was in agreement with data reported by [**Glas *et al.*, (2005)**] in German population and by [**Zhang *et al.*, (2004)**] in Chinese.

HCV infection was reported to be associated by increased markers of oxidative stress. [**Choi *et al.*, (2004)**] found that lipid peroxidation products were increased in serum and liver specimen from hepatitis C patients. Nitric oxide (NO˙) is overproduced in liver cirrhosis and its disturbances seem to play a key role in the pathogenesis of chronic liver disease and are proposed as one of the major endogenous vasodilators in portal hypertension [**Xu *et al.*,(2008)**]. Under conditions of oxidative stress, as seen in certain chronic inflammatory disorders including hepatitis C, reactive NO˙ Species (RNOS), such as peroxynitrite and nitrogen oxides, are currently considered as the main mediators of the deleterious effects to the host of NO., including cytotoxicity and DNA damage [**Zamora *et al.*, (2000)**]. It has been reported that patients with degenerative liver disease had increased lipid peroxide levels in liver tissue and serum [**Seronello *et al.*, (2007)**]. Exposure to more oxidant stress or an inability in the oxidative capacity of the cells might lead to acceleration of peroxidation reactions of some cellular molecules including lipids [**Koruk *et al.*, (2002)**].

In this study patients infected with HCV showed a significant lower mean level of total antioxidant capacity (TAC) and a significant higher level of malonyldialdehyde (MDA), compared to controls. These observations are in agreement with [**Vendemiale *et al.*, (2001) and Aksoy *et al.*, (2003)**].

Moreover, [**Paradis *et al.*, (1997)**] found a direct relationship between fibrosis and liver MDA in patients with HCV infection. **Hassan *et al.*, (2002)**] reported increased NO levels via increased inducible NO· synthase (iNOS) among Egyptian cases. We found that no significant correlation between nitrate levels and age or sex distribution in our cases, these observations are in accordance with **El-Sherif *et al.*, (2008)**] who did not find any significant difference between nitrate levels in patients with chronic active hepatitis and healthy controls among Egyptian subjects. This was also consistent with [**Pârvu *et al.*, (2005)**] among Ramanian and with [**Arkenau *et al.*, (2002)**] among German subjects.

In this study the mean total levels of TAC and MDA showed insignificant difference among the cases with the 3 main genotypes but only NO· level showed a higher significant difference in cases with A2A2 genotype followed by A1A2 and A1A1 genotypes. [**Castellano-Higuera *et al.*, (2008)**] found that cytokine levels contributed to the progression of chronic hepatitis C infection in Spanish population, also oxidative stress and cytokine secretion are closely related to each other's. Inflammatory conditions, such as infection or any situation leading to an acute organic stress, trigger a cytokine response and enhance oxidative stress.

In conclusion, we speculated that subjects having the IL-1Ra A2A2 genotype were having higher risk for getting HCV infection and liver complications whereas those with A1A2 genotype seemed to be more protected against such infections. Also, oxidative stress was documented in HCV cases and was manifested with high levels of MDA and low levels of TAC. In addition, NO· levels were higher among HCV cases with IL-1Ra A2A2 genotype than other cases.

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التعدد الشكلي لجين مضاد المستقبل انترليوكين-١ والأجهاد التأكسدي بين مرضي الإلتهاب الكبدي الوبائي الفيروسي سي .

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يعتبر جين الانترليوكين-١ أول السيتوكين إكتشافاًحيث أنه له دور فعال في التحكم في الجهاز المناعي ضد أي جسم غريب أو أي إصابة بعدوي فيروسية وكذلك في عمليات الأيض التي يقوم بها الكبد.

إن مرض الإلتهاب الكبدي الفيروسي الوبائي سي من أكثر الأمراض شيوعاً في مصر وأغلب دول العالم ويصاحب هذا المرض وجود تغيرات في جهد التأكسد بالجسم وذلك نتيجه وجود الفيروس وما يصاحبه من إلتهابات وتلف في خلايا الكبد.

وفي هذه الدراسة تم إيجاد العلاقه بين التعدد الشكلي للجين الخاص بمضاد المستقبل انترليوكين-١ ومرض الإلتهاب الكبدي الوبائي الفيروسي سي ومدي تأثيره علي الجهد التأكسدي عند هؤلاء المرضي. فقد تم عمل دراسة للحمض النووي للجين الخاص بمضاد المستقبل انترليوكين-١ بأستخدام تفاعل البوليمريز المتسلسل للمرضي المصابين باللإلتهاب الكبدي الفيروسي سي وعددهم ١١٠ شخص من المترددين علي عيادات الأمراض المتوطنة في مستشفي المنصورة الجامعي وايضا علي حوالي ١٢٤ شخص من الأصحاء الذين لا يعانون من وجود أي أمراض وقد تم أخذهم من متبرعيين ببنك الدم بمستشفي الأطفال الجامعي وذلك للمقارنه.

ومن خلال هذه الدراسة فقد وجد أن للجين الخاص بمضاد المستقبل انترليوكين-١ العديد من الطرز الجيني بين المرض المصابين بالإلتهاب الكبدي الوبائي الفيروسي سي وكذلك بين الأفراد الأصحاء. منها الطراز الجيني A1A2 وهو مميز في الأفراد الأصحاء عنه بين المصابين بالفيروس.أما الطراز الجيني A1A1 وكذلك A2A2 فان نسبة ظهوره في مرضي الإلتهاب الكبدي الفيروسي أعلي منها عند الأصحاء.

كما وجد أن أكسدة الدهون والمتمثلة في مركب المالونيل ثنائي الألدهيد فهو أعلي في المرضي عنه في الأصحاء وذلك لوجود فيروس سي وتأثيره علي حالة الجهد التأكسدى في جسم المريض.أما الشق الحر اكسيد النتريك فإنه مميز في حالة الطراز الجيني A2A2 بين مرضي الإلتهاب الكبدي الوبائي الفيروسي سي.

ومن هنا نستنتج أن وجود فيروس سي قد أثر علي الجهد التأكسدى وكذلك علي الجهاز المناعي وان الطراز الجيني A2A2 مميز في مرضي الإلتهاب الكبدي الوبائي الفيروسي سي وأن هناك إحتمالية للحماية من الإلتهاب الكبدي الوبائي الفيروسي سي في وجود الطراز الجيني A1A2.